Characterization of an In Vitro Differentiation Assay for Pancreatic-Like Cell Development from Murine Embryonic Stem Cells: Detailed Gene Expression Analysis.

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Public Summary:

Scientific Abstract:
Abstract Embryonic stem (ES) cell technology may serve as a platform for the discovery of drugs to treat diseases such as diabetes. However, because of difficulties in establishing reliable ES cell differentiation methods and in creating cost-effective plating conditions for the high-throughput format, screening for molecules that regulate pancreatic beta cells and their immediate progenitors has been limited. A relatively simple and inexpensive differentiation protocol that allows efficient generation of insulin-expressing cells from murine ES cells was previously established in our laboratories. In this report, this system is characterized in greater detail to map developmental cell stages for future screening experiments. Our results show that sequential activation of multiple gene markers for undifferentiated ES cells, epiblast, definitive endoderm, foregut, and pancreatic lineages was found to follow the sequence of events that mimics pancreatic ontogeny. Cells that expressed enhanced green fluorescent protein, driven by pancreatic and duodenal homeobox 1 or insulin 1 promoter, correctly expressed known beta cell lineage markers. Overexpression of Sox17, an endoderm fate-determining transcription factor, at a very early stage of differentiation (days 2-3) enhanced pancreatic gene expression. Overexpression of neurogenin3, an endocrine progenitor cell marker, induced glucagon expression at stages when pancreatic and duodenal homeobox 1 message was present (days 10-16). Forced expression (between days 16 and 25) of MafA, a pancreatic maturation factor, resulted in enhanced expression of insulin genes, glucose transporter 2 and glucokinase, and glucose-responsive insulin secretion. Day 20 cells implanted in vivo resulted in pancreatic-like cells. Together, our differentiation assay recapitulates the proceedings and behaviors of pancreatic development and will be valuable for future screening of beta cell effectors.